



the polynucleotide, is cleaved by the nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

80. (New) The method of claim 79 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

81. (New) The method of claim 79 wherein a second oligonucleotide is present during the incubating, wherein the second oligonucleotide hybridizes to a site on the polynucleotide that is in the 3' direction from the site at which the oligonucleotide is hybridized and wherein the second oligonucleotide is substantially non-reversibly hybridized to the polynucleotide under the isothermal conditions.

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82. (New) The method of claim 81 wherein the second oligonucleotide hybridizes to the polynucleotide at a site contiguous with the site on the polynucleotide at which the oligonucleotide hybridizes.

83. (New) The method of claim 82 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of the polynucleotide.

84. (New) A method for detecting a polynucleotide analyte, which comprises:

(a) forming a mixture comprising a sample suspected of containing a polynucleotide analyte, an oligonucleotide and a nuclease,

(b) incubating the mixture at a temperature at which the oligonucleotide reversibly hybridizes to the polynucleotide analyte, wherein the oligonucleotide has a 5' portion which does not substantially hybridizes with the polynucleotide analyte at said temperature and a 3' portion which substantially hybridizes with the polynucleotide analyte at said temperature, thereby forming a polynucleotide complex

comprising at least the polynucleotide analyte and the oligonucleotide, wherein the complex serves as a substrate for the nuclease, and wherein during said incubating the nuclease cleaves the oligonucleotide when the oligonucleotide is hybridized to the polynucleotide analyte to continuously produce (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and includes no more than five nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide analyte, and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide analyte, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

85. (New) The method of claim 84 wherein at least one of the first fragment and the second fragment has a label.

86. (New) The method of claim 84 wherein the first fragment includes no more than one nucleotide from the 5'-end of the portion of the oligonucleotide that substantially hybridizes to the polynucleotide analyte. C

87. (New) The method of claim 84 wherein the mixture further comprises a second oligonucleotide that substantially fully hybridizes to a site on the polynucleotide analyte that is in the 3' direction from the site at which the oligonucleotide hybridizes and wherein the second oligonucleotide is substantially fully hybridized to the polynucleotide analyte at the temperature.

88. (New) The method of claim 87 wherein the second oligonucleotide hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the oligonucleotide hybridizes.

89. (New) A method for detecting a polynucleotide analyte, the method comprising:

(a) providing in combination a medium suspected of containing the polynucleotide analyte, a molar excess, relative to the suspected concentration of the polynucleotide analyte, of a first oligonucleotide at least a portion of which is reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second oligonucleotide that hybridizes to a site on the polynucleotide analyte that is in the 3' direction of the site at which the first oligonucleotide reversibly hybridizes wherein the polynucleotide analyte is substantially fully hybridized to the second oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first oligonucleotide, wherein the first oligonucleotide, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at least a 100-fold molar excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

90. (New) The method of claim 89 wherein the first fragment and/or the second fragment has a label.

91. (New) The method of claim 90 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

92. (New) The method of claim 89 wherein the polynucleotide analyte is DNA.

93. (New) The method of claim 89 wherein the first fragment includes no more than 5 nucleotides from the 5'-end of the portion of the first oligonucleotide that is reversibly hybridizes to the polynucleotide analyte.

94. (New) The method of claim 89 wherein the second oligonucleotide hybridizes to the polynucleotide analyte at a site contiguous with the site on the polynucleotide analyte at which the first oligonucleotide reversibly hybridizes.

95. (New) A method for detecting a DNA analyte, the method comprising:

(a) providing in combination a medium suspected of containing the DNA analyte, a first oligonucleotide at least a portion of which reversibly hybridizes with the DNA analyte under isothermal conditions, a 5' nuclease, and a second oligonucleotide that hybridizes to a site on the DNA analyte that is in the 3' direction from the site at which the first oligonucleotide reversibly hybridizes wherein the DNA analyte is substantially fully hybridized to the second oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing the DNA analyte and the first oligonucleotide under the isothermal conditions, wherein the first oligonucleotide, when hybridized to the DNA analyte, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the DNA analyte and (ii) a second fragment that is 3' of the first fragment with reference to the intact first oligonucleotide and is substantially hybridizable to the DNA analyte, wherein at least a 100-fold molar excess, relative to the DNA analyte, of the first fragment and/or the second fragment is produced, and wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the DNA analyte.

96. (New) The method of claim 95 wherein the first oligonucleotide has a substituent that facilitates separation of the first fragment or the second fragment from the medium.

97. (New) The method of claim 95 wherein first fragment and/or second fragment has a label.

98. (New) The method of claim 97 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

99. (New) The method of claim 95 wherein the second oligonucleotide hybridizes to the DNA analyte at a site contiguous with the site on the DNA analyte at which the first oligonucleotide reversibly hybridizes.

100. (New) The method of claim 95 wherein the first oligonucleotide and/or the second oligonucleotide is DNA.

101. (New) A method for detecting a polynucleotide analyte, the method comprising:

(a) providing in combination a medium suspected of containing the polynucleotide analyte, a first DNA oligonucleotide at least a portion of which reversibly hybridizes with the polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second DNA oligonucleotide that hybridizes to a site on the polynucleotide analyte that is 3' of, and contiguous with, the site at which the first DNA oligonucleotide reversibly hybridizes, wherein the polynucleotide analyte is substantially fully hybridized to the second DNA oligonucleotide under the isothermal conditions,

(b) reversibly hybridizing under the isothermal conditions the polynucleotide analyte and the first DNA oligonucleotide, wherein the first DNA oligonucleotide, when hybridized to the polynucleotide analyte, is cleaved by the 5'-nuclease as a result of the presence of the polynucleotide analyte to provide, in at

least a 100-fold molar excess of the polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte and/or (ii) a second fragment that is 3' of the first fragment with reference to the intact first DNA oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein said first fragment and/or said second fragment is/are continuously produced under said isothermal conditions, and

(c) detecting the presence of the first fragment, the second fragment, or the first and second fragments, the presence thereof indicating the presence of the polynucleotide analyte.

102. (New) The method of claim 101 wherein the first fragment and/or the second fragment has a label.

103. (New) The method of claim 102 wherein the label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

104. (New) The method of claim 101 wherein the polynucleotide analyte is DNA.

105. (New) A method for modifying an oligonucleotide, the method comprising incubating the oligonucleotide with a polynucleotide and a 5'-nuclease under isothermal conditions, wherein at least a portion of the oligonucleotide reversibly hybridizes to the polynucleotide under said isothermal conditions and wherein the oligonucleotide, when the portion is hybridized to the polynucleotide, is cleaved by the 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than five nucleotides from the 5'-end of the portion and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby modifying said oligonucleotide, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions.

106. (New) A method for producing oligonucleotide cleavage products from an enzyme catalyzed cleavage of the oligonucleotide, the method comprising:

(a) combining, in any order, a polynucleotide, an oligonucleotide having a 3'-portion which substantially hybridizes with the polynucleotide and a 5' portion which does not substantially hybridize with the polynucleotide, and a nuclease, wherein the oligonucleotide, when hybridized to the polynucleotide, forms a polynucleotide complex comprising at least the polynucleotide and the oligonucleotide, the complex serving as a substrate for the nuclease,

(b) incubating the oligonucleotide, the polynucleotide and the nuclease at a temperature at which the oligonucleotide reversibly hybridizes to the polynucleotide, wherein the nuclease cleaves the oligonucleotide when the oligonucleotide is hybridized to the polynucleotide to continuously produce at said temperature (i) a first fragment that is substantially non-hybridizable to the polynucleotide and includes no more than 5 nucleotides from the 5'-end of the portion which substantially hybridizes to the polynucleotide, and (ii) a second fragment that is 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide, thereby producing oligonucleotide cleavage products.

107. (New) A method for detecting a polynucleotide analyte, which comprises:

(a) reversibly hybridizing an oligonucleotide with a polynucleotide analyte and a 5'-nuclease under isothermal conditions wherein the polynucleotide analyte serves as a recognition element to enable the 5'-nuclease to cleave the oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to the polynucleotide analyte, and (ii) a second fragment that lies 3' of the first fragment with reference to the intact oligonucleotide and is substantially hybridizable to the polynucleotide analyte, wherein at least a 100-fold molar excess of the first fragment and/or the second fragment are obtained relative to the molar amount of the polynucleotide analyte, wherein said first fragment and said second fragment are continuously produced under said isothermal conditions, and